

1 **Title:**

2 Faecal particle size in free-ranging proboscis monkeys, *Nasalis larvatus*: Variation between
3 seasons

4
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28 **Abstract**

29 Reducing the size of food particles is crucial for herbivores. Seasonal dietary changes are
30 known to influence animals' chewing efficiency. Proboscis monkeys (*Nasalis larvatus*) are
31 foregut fermenters, with a high chewing efficiency allowing them to achieve very fine faecal
32 particles. In this study, we investigated how proboscis monkeys' chewing efficiency varies
33 among wet and dry seasons, hypothesising differences possibly related to diet change. Faecal
34 particle size analysis is an established approach to estimate chewing efficiency in mammalian
35 herbivores. We analysed 113 proboscis monkey faecal samples collected in the Lower
36 Kinabatangan Wildlife Sanctuary, between 2015 and 2017. By following standard sieve
37 analysis protocols, we measured a mean particle size $MPS_{(0.025-8)}$ of 0.45 ± 0.14 mm, and
38 confirmed a previous result that proboscis monkeys have a very low faecal MPS. This study
39 highlights a seasonal influence on proboscis monkeys' chewing efficiency, with smaller MPS
40 (better chewing efficiency) during the wet season. During that time of the year, individuals
41 may potentially change their diet, as all faecal samples contained intact seeds. Whether the
42 seasonal MPS difference in proboscis monkeys is smaller than in other colobines due to their
43 'rumination' strategy remains to be investigated.

44

45 **Keywords**

46 *Nasalis larvatus*, chewing efficiency, nutritional ecology, forestomach, diet change, foregut
47 fermenter

48

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50

51 **Introduction**

52 Reducing the size of food particles is crucial in herbivores that rely on gut microbiota
53 to digest plant components. In herbivores, fibre digestion relies on digesta retention and
54 particle size. To accomplish the same digestibility, large digesta particles will need longer
55 retention times than smaller ones [Bjorndal et al., 1990]. Measuring faecal particle size by wet
56 sieving analysis, is an established non-invasive approach to determine the chewing efficiency
57 of mammals [Fritz et al., 2009]. Several studies focused on faecal particle size in mammals
58 [Fritz et al., 2009; Clauss et al., 2015], or more specifically in ruminants [Renecker and
59 Hudson, 1990; Clauss et al., 2002] and primates [Dunbar and Bose, 1991; Matsuda et al.,
60 2014; Venkataraman et al., 2014; Weary et al., 2017]. Across mammals, the size of faecal
61 particles usually increases with animal body mass [Fritz et al., 2009]. However, among
62 primates, the proboscis monkey (*Nasalis larvatus*) displays a particularly small mean particle
63 size (MPS) for its average body mass (15 kg) [Matsuda et al., 2014]. Proboscis monkeys are
64 foregut fermenters [Matsuda et al., 2014]. Like other colobine primates, they have a
65 sacculated forestomach where the food is fermented [Bauchop, and Martucci, 1968; Milton,
66 1993]. Regurgitation and remastication (i.e. rumination) has been observed in wild proboscis
67 monkeys [Matsuda et al., 2011a]. Whether this facultative rumination strategy explains how
68 proboscis monkeys achieve particularly fine faecal particles remains unclear [Matsuda et al.,
69 2014].

70 Seasonal dietary change and dental wear are known to influence animals' chewing
71 efficiency [Venkataraman et al., 2014]. Within a species, faecal particle size can vary in
72 relation to diet [Renecker and Hudson, 1990] or seasons [Nygren and Hofmann, 1990]. For
73 instance, in gelada baboons (*Theropithecus gelada*), chewing efficiency decreases during the
74 dry season when individuals feed on tougher non-preferred food items, with a more distinct
75 effect in older individuals [Venkataraman et al., 2014]. The opposite is observed in

76 frugivorous chimpanzees (*Pan troglodytes schweinfurthii*): MPS is higher when chimpanzees
77 feed on drupe fruits (preferred foods) than on figs (non-preferred foods) [Weary et al., 2017].
78 The authors suggested that chewing efficiency might be less critical in frugivores than in
79 typical folivores, because they did not observe an effect of age on MPS.

80 With their natural diet, proboscis monkeys are excellent candidates to investigate how
81 MPS might change throughout the year. While proboscis monkeys were first considered
82 essentially folivores, it is now recognised that they preferentially feed on unripe fruits/seeds
83 when they are available [Matsuda et al., 2009]. The present study investigates how proboscis
84 monkeys' chewing efficiency varies among wet and dry seasons. We hypothesised that
85 proboscis monkeys will achieve a higher chewing efficiency (MPS will decrease) during the
86 season when individuals are able to consume their preferred food.

87 Moreover, we investigated some methodological aspects of sieve analysis. Extending
88 the sieve column (adding larger top or smaller bottom sieves) is known to influence MPS
89 measurements [Fritz et al., 2012], as well as including the weight of unchewed items (i.e.
90 large seeds) and maximum particle length (MPL) in the MPS calculation [Weary et al., 2017].
91 Therefore, we combined various sets of sieves, with or without the MPL, and assessed the
92 impact on MPS measurements.

93

94 **Material and Methods**

95 Study site

96 Our study took place in the Lower Kinabatangan Floodplain (5°20'-05°45' N, 117°40'-
97 118°30' E), in Eastern Sabah (Malaysian Borneo), between 2015 and 2017. Daily
98 temperatures and rainfall were measured at the research station. Below we will refer to dry
99 season (May-June-July) where the mean monthly rainfall is 120 (\pm SD=100) mm and to the

100 wet season (November-February) where it reaches 243 (\pm SD=104) mm. Mean minimum and
101 maximum temperatures reached 24.4 (\pm SD=0.6) and 30 (\pm SD=1.7) °C, respectively.

102

103 Faecal sampling

104 In riverine forests, proboscis monkeys are known to take refuge along riverbanks to spend the
105 night [Matsuda et al., 2011b]. During this study, we conducted boat-based surveys along the
106 Kinabatangan River, in the late afternoon, to find proboscis monkey groups settled at their
107 sleeping sites. To avoid sampling the same group multiple times, we searched for proboscis
108 monkey groups in different parts (North and South riverbanks) along a pre-established 21 km
109 transect in a month. In the morning, we travelled back to the group's location of the previous
110 evening. Once the group left the riverside to forage further inland, we moved to the riverbank
111 to search for fresh faecal samples that had fallen under sleeping trees. We collected large
112 samples, presumed to belong to adult individuals (undistinguished sex). Between May and
113 July 2015, January and February 2016, and November 2016 and February 2017, two faecal
114 samples were collected per group and placed in separate tubes, to perform two different
115 analyses: manual and wet sieving analyses. 137 samples (15 ± 5 samples/month) were
116 analysed by the manual method and 113 faecal samples (13 ± 6 samples/month) by wet
117 sieving method.

118

119 Manual analyses

120 Faecal samples were cleaned with water in a 0.4 mm mesh strainer to discard faecal matter.
121 The remaining digested items were searched for intact seeds. Percentages of samples
122 containing seeds were used to assess seasonal changes.

123

124 Wet sieving analyses

125 Faecal samples were stored in a tube with 70% ethanol [Matsuda et al., 2014]. They were
126 analysed using the standard wet sieving method [Fritz et al., 2012]. Before sieving, each
127 sample was suspended in a beaker filled with water that was stirred continuously for 12 hours.
128 The sample was then poured over a series of 10 sieves with mesh size of 8, 4, 2, 1, 0.5, 0.25,
129 0.125, 0.63, 0.04 and 0.025 mm (Retsch AS 200 digit, Haan, Germany). We conducted the
130 sieving analysis for 10 minutes with an amplitude of 2 mm and a water flow of approximately
131 2 l/min. If a particle was retained on the largest sieve, its size was recorded as the maximum
132 particle size. Particles retained on each sieve were transferred onto pre-weighed petri dishes
133 and dried at 103°C overnight. After cooling in a desiccator, petri dishes were weighed with an
134 analysis balance with measuring accuracy of 1 mg (Kern AEJ 220-4M, Kern, Balingen,
135 Germany). When large seeds (≥ 2 mm) were retained intact in sieves, they were removed,
136 weighed and subtracted from the respective sieve weight. However, the smaller (< 2 mm) and
137 numerous seeds, such as *Ficus* and *Nauclea* seeds, were logistically impossible to remove
138 from the analysis [Weary et al., 2017].

139

140 Among various indices, the discrete mean has been proposed as a standard to describe the
141 MPS value obtained from sieving analyses [Fritz et al., 2012]. To compare our results with a
142 previous study conducted on proboscis monkey's faecal particle sizes [Matsuda et al., 2014],
143 we excluded the two smallest sieves (mesh sizes: 0.040 and 0.025 mm) from the MPS
144 calculation, as they were not used by Matsuda et al. [2014]. Although the latter study used a
145 larger top sieve (16 mm) than we did, no particles were ever retained on it.

146

147 Statistical analyses

148 We carried out t-tests on log-transformed data to compare $MPS_{(0.025-8mm)}$ values between dry
149 and wet seasons. We compared MPS values calculated for 28 faecal samples, with or without

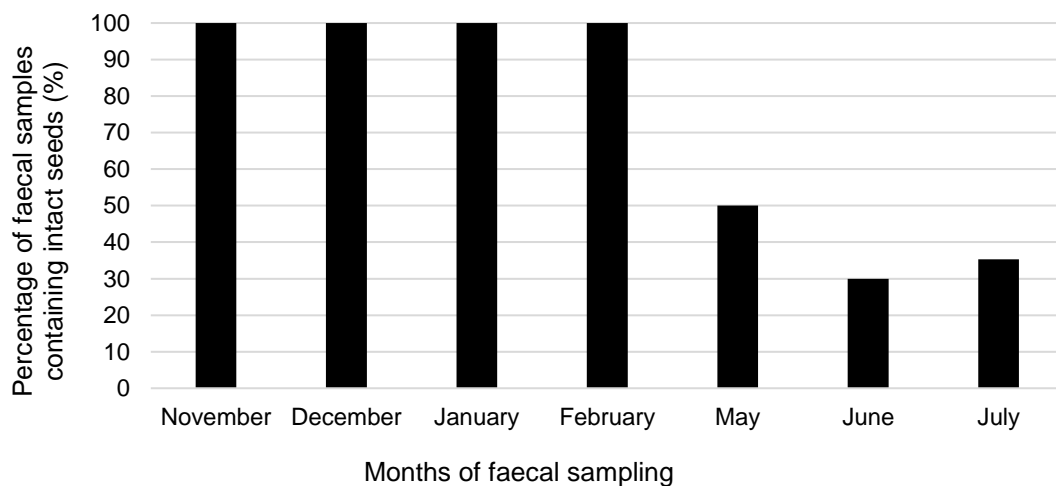
150 considering the length of the maximum particles and using series of 10 (0.025-8 mm) or 8
151 (0.063-8 mm) sieves. To assess the difference between those four MPS measurements, we
152 performed a related-samples Wilcoxon signed rank test between all pairs of MPS values,
153 using the Bonferroni adjustment (for multiple comparisons). R 3.4.0 [R Development Core
154 Team, 2016] was used for all statistical analyses, with statistical significance of $p < 0.05$.

155

156 **Results**

157 **Manual analyses**

158 By cleaning fresh faeces (N=137), we observed that the percentage of faecal samples
159 containing intact seeds changed throughout the year (Fig. 1), with a mean of $100 \pm 0 \%$ during
160 the wet season (November-February) and of $38 \pm 10 \%$ during the dry season (May-July).



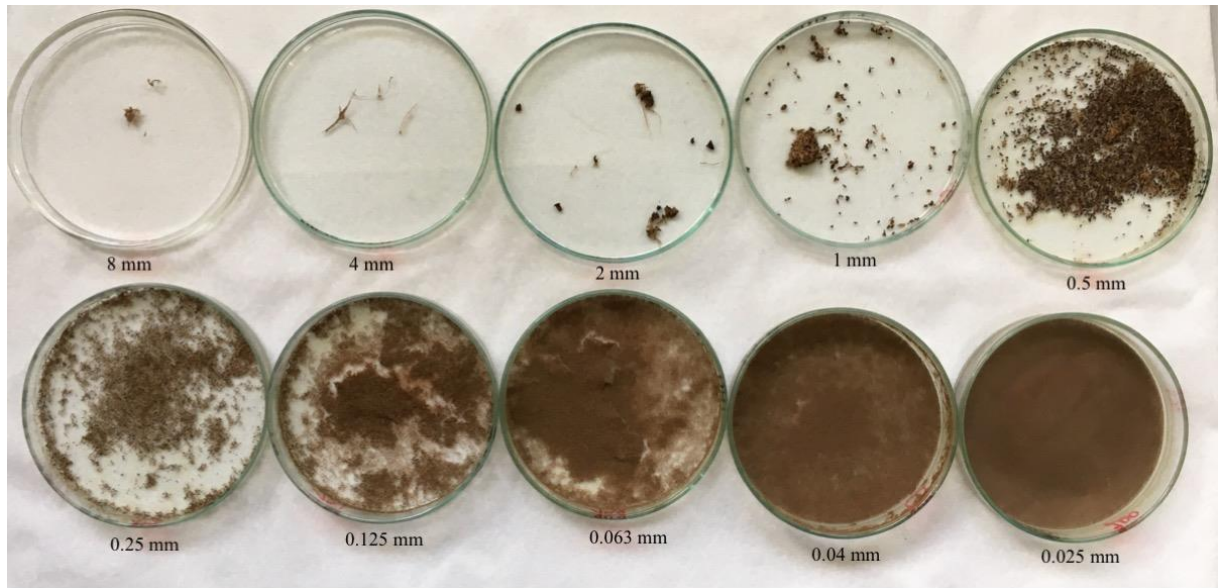
161

162 Figure 1 Percentage of faecal samples of proboscis monkeys (*Nasalis larvatus*) collected
163 during different months that contain intact seeds

164

165 **Wet sieving analyses**

166 Fig. 2 illustrates the typical way faecal particles are distributed after wet sieving analysis.



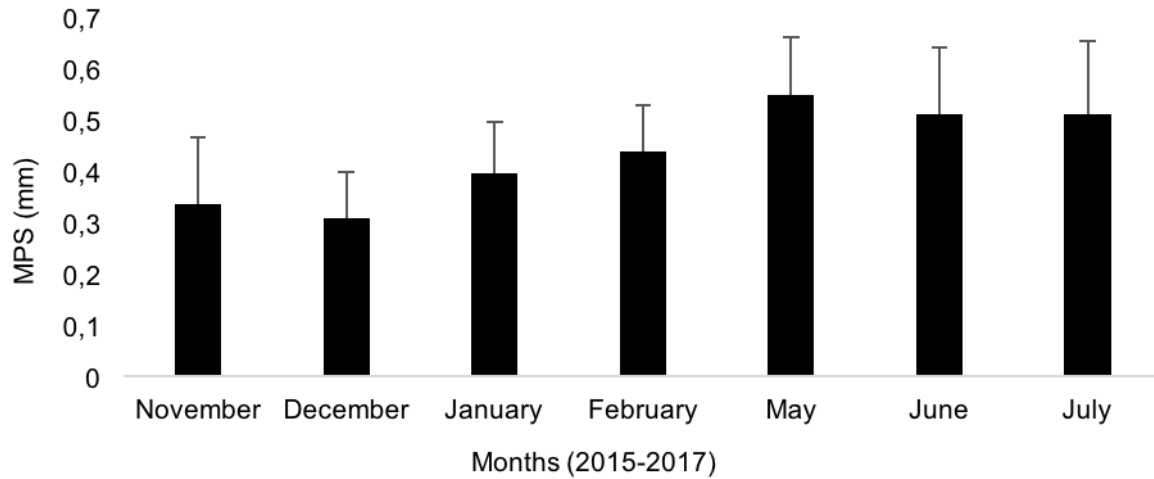
167

168 Figure 2. Distribution of proboscis monkey (*Nasalis larvatus*) faecal particles after wet
169 sieving analysis. Each petri dish represents one of the 10 cascade sieves, ordered from 8 mm
170 until 0.025 mm mesh size. The sample PMF217-1 collected on the 28th of January 2017 was
171 used for this example.

172

173 By using a cascade of 10 sieves (mesh sizes: 8 to 0.025 mm), the MPS reaches an average of
174 0.45 ± 0.14 mm and increases over the course of the observation period from November to
175 July (Fig. 3). By using a series of eight sieves (mesh sizes: 8 to 0.063 mm) like in Matsuda et
176 al. (2014), we observe that $MPS_{(0.063-8mm)}$ is significantly larger than $MPS_{(0.025-8mm)}$, reaching
177 0.55 ± 0.14 mm ($V=6441$, $N=113$, $p<0.001$).

178

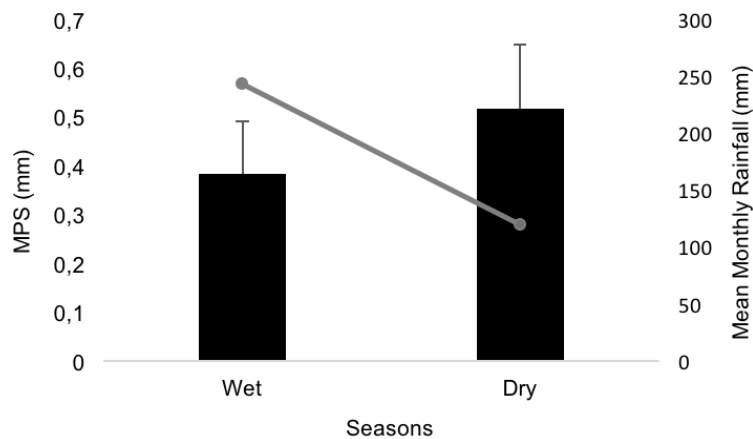


179

180 Figure 3. Variation in proboscis monkey (*Nasalis larvatus*) MPS over the course of the
 181 observation period (November-July)

182

183 When comparing MPS_(0.025-8mm) of proboscis monkeys across seasons, we observe that
 184 MPS is significantly smaller during the wet season than the dry season (MPS_{wet} = 0.38 ± 0.11
 185 mm, MPS_{dry} = 0.52 ± 0.13 mm; t-test t = -6.2812, p < 0.001) (Fig. 4).



186

187 Figure 4. MPS variation of proboscis monkeys (*Nasalis larvatus*) across seasons

188

189 Table 1 summarises four MPS measurements. Using a series of 10 sieves (with both smallest
 190 mesh sizes 0.040 and 0.025 mm) resulted in finer MPS than calculation with the 8-sieve
 191 cascade. MPS measurements were significantly larger when including the maximum particle
 192 length (MPL) for the largest sieve in the calculation.

193

194 Table 1. Mean particle size \pm SD of 28 faecal samples of proboscis monkeys (*Nasalis*
195 *larvatus*) measured using two series of sieves (indicated by the sieve size of the smallest and
196 largest sieves) and with or without taking the maximum particle length (MPL, when particles
197 were retained on the largest sieve) into account in the MPS calculation

Method	MPS \pm SD (mm)
0.025-8 MPL	0.48 \pm 0.11
0.025-8	0.47 \pm 0.11
0.063-8 MPL	0.59 \pm 0.11
0.063-8	0.57 \pm 0.10

198

199 Discussion

200 This study focused on proboscis monkeys' faecal samples collected between 2015 and
201 2017, during wet and dry seasons. We confirm the very small discrete mean faecal particle
202 size in proboscis monkey (MPS_(0.025-8mm) = 0.45 \pm 0.14 mm), for its average body mass. We
203 measured a MPS_(0.063-8mm) of 0.55 \pm 0.14 mm, similar to the results of a previous study
204 (MPS_(0.063-16mm) = 0.53 \pm 0.09 mm) obtained by analysing 10 samples collected in June-July
205 2010 [Matsuda et al., 2014]. The fine MPS indicates the generally high chewing efficiency of
206 proboscis monkeys. For example, in comparison, frugivorous primates in Borneo, such as
207 macaques (*Macaca fascicularis* and *M. nemestrina*) and orangutans (*Pongo pygmaeus*), have
208 larger MPS_(0.063-16mm) respectively ranging from 1.07 \pm 0.47 to 2.30 \pm 0.78 mm [Matsuda et
209 al., 2014].

210 We observed that MPS is even smaller during the wet season than the dry season. The
211 same pattern had been observed in folivorous gelada baboons [Venkataraman et al., 2014].
212 The latter showed a lower MPS in wet season when consuming less tough food items. In our
213 study, the MPS difference might also be linked to a change in diet. Smaller MPS values
214 during the wet season correlate with high percentage of intact seeds in faeces, suggesting

215 individuals might consume more fruits and their seeds. However, this assumption must be
216 considered carefully, as the absence of seeds in faeces does not always imply that individuals
217 did not eat fruits (i.e. seeds could be totally digested, chewed or discharged). Mismatches
218 have been observed between proboscis monkey fruit feeding activity and seeds detected in
219 faeces [Matsuda et al., 2013]. Primates, including proboscis monkeys, usually avoid feeding
220 on tough leaves or leaf parts [Hill and Lucas, 1996; Teaforde et al., 2006; Dunham and
221 Lambert, 2016; Matsuda et al., 2017]. The same pattern is observed in chimpanzees where
222 fallback foods are significantly tougher than preferred items (fruits) [Vogel et al., 2008].
223 However, in Bornean orangutans (*P. p. wurmbii*), mechanical properties of leaves and fruits
224 did not vary significantly [Vogel et al., 2008]. In the present study, we did not analyse the
225 toughness of food items fed on by proboscis monkeys. However, in comparison to leaves,
226 fruits and seeds are generally considered as high quality food [Milton, 1993; Hanya and
227 Bernard, 2015]. Containing less fibre, fruits generally are more digestible than leaves [Milton,
228 1993]; consumption of unripe fruits may lead to smaller MPS. Further work should
229 investigate nutritional and mechanical properties of unripe fruits and their seeds consumed by
230 the proboscis monkey to better understand the feeding selection in this endangered primate.

231 As in gelada baboons, we suggest here that fallback food consumption during some
232 parts of the year leads to a reduction of chewing efficiency which might potentially negatively
233 impact the animals' fitness [Venkataraman et al., 2014]. There is preliminary evidence that
234 'rumination' activity in proboscis monkeys is higher during times of increased leaf
235 consumption [Matsuda et al., 2014], which could potentially attenuate the change in MPS
236 associated with leaves. If this was a general pattern, then the MPS difference between the
237 seasons obtained in the present study should be of a lower magnitude than in other arboreal
238 primates that show a seasonal foraging pattern but do not 'ruminate'. Compared to geladas
239 with a seasonal MPS difference of 0.3-0.4 mm in prime adults, the proboscis monkeys of the

240 present study did show a lower difference (0.14 mm, Fig. 4). However, due to diet differences
241 between hindgut fermenter geladas and foregut fermenter proboscis monkeys, this
242 comparison should be treated cautiously. We suggest further studies should determine
243 whether proboscis monkey individuals achieve finer MPS when they ‘ruminate’ as opposed to
244 times when they do not. Such data could help unravel the relevance of facultative rumination
245 as a response to diet constraints. Finally, future research should also investigate if seasonal
246 changes in MPS are also measured in other colobine primates, as data is missing so far.

247

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255

256

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